OBSERVATIONS IN NONSPECIFIC IMMUNITY

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In analyzing the data collected in the medical examinations of students entering the University of Wisconsin during a period of 4 years, Van Valzah,[1] in 1914, reported that in this group of over 5,000 persons, the women gave a previous history of considerably greater percentage morbidity than the men in all the more common communicable diseases. The figures are given in table 1.

"In order to understand the more frequent occurrence of these diseases in the women, a study was made of the home environment of the various students and it was found that a large majority of the women came from towns and cities while a large proportion of the male enrollment came from rural districts." That the previous environment was an important factor in the striking difference in incidence of disease in the two sexes was further emphasized by figures obtained from observing the students during their 4 years of college life. The proportion of men to women in the University was about 2½ to 1 whereas the morbidity relation in these diseases during University life was much greater than this, showing a ratio of 7 for the men to 1 for the women, a reversal of the figures for precollege life. The influence of previous city life is still further shown by the high incidence of communicable diseases among the "short course" agricultural students. This group of students, practically all of whom come from rural districts, made up at that time about 10% of the total student body and yet they showed a 31% incidence from these diseases.

Year after year, the figures in the university tell the same story. One year it is measles, the next mumps and the next possibly a streptococcus epidemic, but always if an outbreak occurs, the incidence is higher among the short course students than in the rest of the student body. Not only is the incidence greater among the men from strictly rural communities, but also when they are ill their illness is of greater severity, as a rule, than is that of the regular students. In epidemics when the temperature averages 102 F. the short course students commonly have a temperature of 103-104 F., and present a more severe

Received for publication, May 4, 1922.

[1] Wis. Med. Jour., 1915, 14, p. 1.

clinical picture.[2] During the influenza epidemic of 1918,[3] for example, the death rate for this group was 3.21% while for the rest of the student body, including the S. A. T. C., the death rate was 0.82%.

This same point has been brought out on a much larger statistical basis by Love and Davenport [4] and by Vaughan and Palmer [5] as a result of their findings in the army cantonments during the recent war. These observers have noted that not only was the morbidity and mortality from the common infectious diseases greater among the men from the sparsely settled rural communities, but this was also true for the less common diseases, such as epidemic meningitis and pneumonia. Except for some slight irregularities, the admission rate for measles. mumps, scarlet fever, primary lobar pneumonia and epidemic menin-

TABLE 1

INCIDENCE OF COMMUNICABLE DISEASES IN STUDENTS BEFORE ENROLLMENT IN UNIVERSITY

Number of Students	Measles, Percentage	Whooping Cough, Percentage	Mumps, Percentage	Chicken Pox, Percentage
Men.........................3,955	79	46	60	40
Women......................1,780	92	67	65	63
Total.....................5,735				

gitis was in approximately inverse ratio to the density of the population in the regions from which the troops came. This is shown most strikingly, for example, in the number of cases of measles occurring in 1917 at Camp Wheeler, Ga., and at Camp Wadsworth, S. C. These camps were only 100 miles apart and located in the same general type of country. Measles was present at both camps, yet at Camp Wheeler the mean annual admission rate was 500 per 1,000 while at Camp Wadsworth it was about one in 1,000. An examination as to the homes of these troops showed that the soldiers at Camp Wheeler were recently recruited National Guard troops from Virginia, Alabama and Florida, whereas the men at Camp Wadsworth were seasoned National Guard troops from New York City and its vicinity.

It would seem to be an epidemiologic fact, therefore, that when persons from rural communities are brought suddenly into close associa-

[2] Middleton, W. S., and Van Valzah, R.: Personal communication.

[3] Van Valzah, R.: Influenza Among University Students, Report to Board of Regents, University of Wisconsin, 1918.

[4] Arch. Int. Med., 1919, 24, p. 129.

[5] Military Surgeon, 1920, 46, p. 1.

tion with many persons, they exhibit a lower resistance to the ordinary communicable diseases than do their city bred brethren.

There are several obvious explanations for this fact. Doubtless the most important factor, in the greater resistance of the "city dwellers," is, as Van Valzah pointed out, an actual specific immunity acquired during the course of recovery from recognized cases of measles, whooping cough, chicken pox, etc. The incidence of such acute diseases is in most parts of the United States higher among city children [4] than among the children in the farming communities. In table 2, are figures obtained from Dr. I. F. Thompson of the Wisconsin State Board of Health, which show that in two of the less common communicable diseases also, namely, typhoid fever and pneumonia, the mortality, and

TABLE 2

DEATH RATE IN WISCONSIN RURAL AND URBAN COMMUNITIES, URBAN BEING INTERPRETED AS POPULATIONS OF 2,500 OR MORE

	1910		1911		1912		1913		1914	
	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural
Typhoid fever rates per 10,000 population	3.5	2.0	1.8	0.9	1.8	0.8	2.4	0.6	0.9	0.2
Pneumonia rates per 10,000 population	[illegible].1	5.3	15.9	7.6	12.4	5.7	12.6	5.9	Not obtained	Not obtained

undoubtedly the morbidity as well, has been lower in the rural communities of Wisconsin than in those of over 2,500 inhabitants, considered as urban. It is recognized that in the case of typhoid fever, this relation between urban and rural districts would not hold throughout the country, as this disease is one which in many parts shows a higher incidence in the rural districts. In Wisconsin, the high rate for urban communities has been largely due to the conditions prevailing in the cities situated on Lake Michigan, where that body of water is used both as a source of water supply and as a means of sewage disposal.

One of the difficulties in obtaining adequate figures relative to morbidity and mortality is that the U. S. Census Bureau has used 10,000 as the arbitrary division between rural and urban communities, and this point of division has been generally adopted. We feel that this is not a suitable dividing line as many communities with a population of 4,000-5,000 have the disadvantages of the close associations of city life without the advantages, such as an adequately supervised public water supply, sewage disposal, etc.

Aside from the greater incidence of recognized cases in city communities, there is also the undoubted occurrence of unrecognized mild attacks. The possibility of a higher incidence of such abortive attacks is obviously greater in the more closely packed communities than in rural districts. This also would tend to produce specific immunity to the common communicable diseases.

Zingher [6] reports an interesting instance of specific immunity in a densely populated urban district as contrasted with a less densely populated area. He states that the number of positive Schick tests was definitely greater among the children from the homes of the more well-to-do of New York than among the children of the community in general and that the lowest number of positives was found among the children of the slums. Presumably, a comparison of city and country communities would show an even greater contrast.

Furthermore, the higher mortality rate in densely populated city districts may result in a certain amount of natural selection of those best fitted to survive in a rich and varied bacterial environment, the weaker members failing to reach maturity. This may well be a factor in diseases in which the mortality is high, but it is hard to see how it would play any considerable part in a disease like mumps, for example, in which fatalities among children are practically nil.

The factor of specific immunity, then, is admittedly the most important point in rendering adult city dwellers more resistant than their rural brothers when brought in contact with infectious agents. Is it not possible, however, due to the greater interchange of organisms and the higher incidence in general of communicable diseases in our cities that a nonspecific immunity of some degree may be developed? Is there a nonspecific immunity entirely apart from the well recognized "group reactions?" Through repeated slight injuries to the antibody producing cells, should we not expect on a pathologic basis a hyperplasia of these tissues, an actual extension of the lymphoid tissue or the bone marrow for example? Such a training of the antibody-producing mechanism should result either in a more rapid response in the production of antibodies or in the production of more antibodies as the result of a given stimulus.

The greatest emphasis on nonspecific factors in resistance has come largely from clinical medicine. Nonspecific bacterial vaccines, milk, normal serum, protein-split products, enzymes, tissue extracts as well as colloidal metals have all been employed in recent years in the treatment of almost every com-

[6] Jour. Amer. Med. Assn., 1921, 77, p. 835.

municable disease but more especially in the treatment of different types of arthritis and typhoid fever. Auld in Great Britain, Chantemesse in France, R. Kraus and Ludke in Germany, and J. L. Miller in this country are among the leading exponents of this type of therapy and have all reported numerous successful results in its application.

From the laboratory side, the finding by Vaughan and Wheeler[7] of apparently the same toxic elements by the hydrolytic cleavage of either pathogenic or nonpathogenic bacteria or from altogether nonbacterial sources is of great importance in this connection. V. C. Vaughan, Jr.,[8] succeeded in building up a nonspecific resistance in experimental animals by several injections of this toxic element. These animals rarely, however, withstood more than a single fatal dose whereas with the nontoxic specific fraction, an active immunity could be induced so that such animals would withstand many fatal doses. Animals immunized with the nonspecific elements did not show any demonstrable immune bodies.

Bacteriologists have always delighted in correlating this work of Vaughan with the splitting of protein through the use of alexin and specific antibody (Friedberger, Friedman, etc.) resulting in the production of nonspecific toxic substances commonly known as anaphylatoxins. These two lines of work have furnished support to the contention that difference in clinical picture is due largely to such factors as location of the lesion, invasive properties of the bacteria, etc., and that specific endotoxins do not exist .

. Jobling and Petersen,[9] approaching the problem from another angle, have, in a series of papers, demonstrated the mobilization of nonspecific proteases and lipases in the blood as important factors in resistance.

Hektoen,[10] Bieling,[11] Pinner and Ivancevic,[12] and Hermann[13] have all shown that the injection of a second and different protein will call forth an increased amount of antibodies against the antigen first used in immunization.

We have approached the subject from still another point of view, that of the striking differences in morbidity observed year after year among the various groups of students at the University of Wisconsin. The following experiments were planned, therefore, to reproduce, if possible, some of the conditions prevailing among persons living in urban and rural surroundings with the hope of obtaining some suggestions in answer to the questions stated in the foregoing

Twelve rabbits were selected, weighed, bled—3 or 4 c c from each animal—for a normal serum sample and then placed in individual cages. They were divided into 2 groups, the first (1-6) to be made into "city dwellers" by a series of vaccinations, and the second (7-12) to remain under the same conditions as to care and feeding, but to receive no treatment, and therefore to be called "country dwellers." We

[7] Protein Split Products, 1913.
[8] Jour. Med. Research, 1905, 14, p. 67.
[9] Arch. Int. Med., 1917, 19, p. 1042.
[10] Jour. Infect. Dis., 1917, 21, p. 219.
[11] Ztschr. f. Immunistätsf., 1919, 33, p. 246.
[12] Ibid., 1920, 30, p. 542.
[13] Jour. Infect. Dis., 1918, 23, p. 457.

planned then to inoculate both groups of animals with an antigen differ ing widely from the vaccines used for the city dwellers and to titrate the serum of each animal for antibodies developed against this second antigen over a period of 3 weeks.

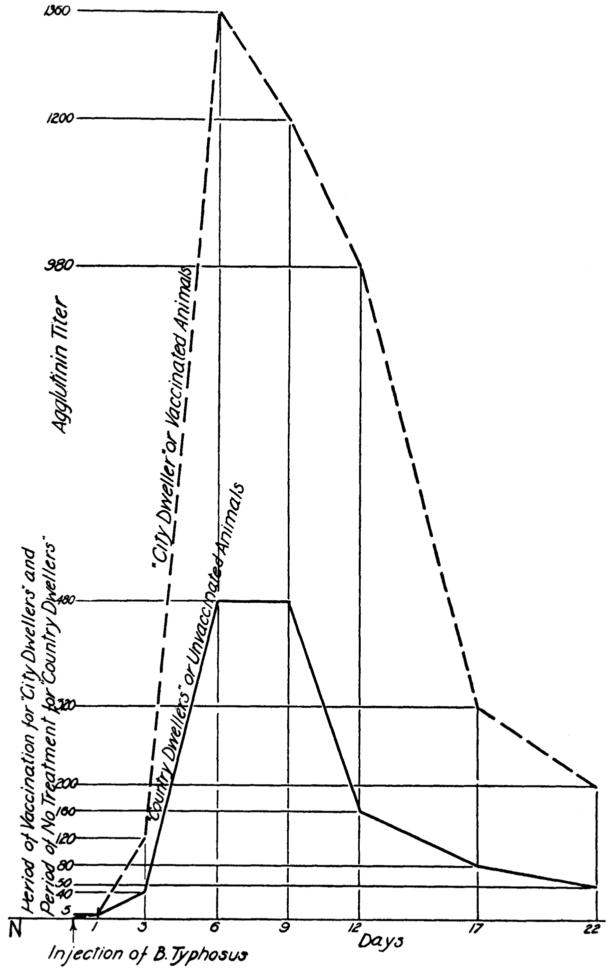

Chart 1.—Agglutination curves of vaccinated and unvaccinated animals, intravenous series.

Vaccines sufficient for the whole series of injections were made with 5 organisms, 2 different strains of Streptococcus viridans, 1 hemolytic streptococcus, a Staphylococcus aureus and a pneumococcus, type undetermined. All of these organisms had been recently isolated.

These particular species of bacteria were chosen beacuse of their prevalence in many infections in the human upper respiratory tract. Salt solution suspensions were made from 24-hour cultures grown on Löffler's blood serum and then sealed in glass and heated to 63-65 C. for 50 minutes.

Subsequent plantings showed that all vaccines were sterile except that a few colonies grew from one of the viridans vaccines. All suspensions were made up to have an opacity equal to that of an equal thickness of a 0.3% lecithin solution.

The first group of animals (rabbits 1-6) was injected intravenously at 5 day intervals with 1 c c of each vaccine separately as follows:

17-3-21	Staphylococcus aureus (Stock culture)
22-3-21	Streptococcus hemolyticus (Sprague)
26-3-21	Streptococcus viridans (Robertson)
1-4-21	Pneumococcus (Young)
6-4-21	Streptococcus viridans (Clark)

A rest of eleven days was then allowed during which the M. L. D. of B. typhosus was determined. This organism was chosen because of its marked differences from the organisms used for the preliminary vaccinations, so that no possibility of group reactions, as that term is ordinarily used, could enter into the problem. The organisms used for vaccination were all cocci, the secondary antigen was a bacillus. The cocci were all gram-positive, B. typhosus is gram-negative, with all the essential differences in protein that that staining reaction implies. The cocci used all stimulate the bone marrow to produce a polymorphonuclear leukocytosis, whereas the typhoid bacillus produces a lymphotoxin.

The B. typhosus used was an old stock culture and relatively non-virulent. As a result of 2 series of injections, the M. L. D. was found to be approximately three 24-hour slant agar cultures. Accordingly, a dosage of 2 cultures was determined on.

On the twelfth day after the last injection of cocci, therefore, both the vaccinated and the unvaccinated groups of animals were injected intravenously with B. typhosus. In order to prolong the injuring effect, the dose was divided and one culture of the living organisms given on that day, and one on the succeeding day.

One of the "city dwellers" (rabbit 6) died during the night following the first injection of B. typhosus and one (No. 4) was eliminated from the series because of an extensive subcutaneous staphylococcus abscess which was causing considerable loss of weight. Within 15 hours

after the second injection of B. typhosus rabbits 7, 8, 9, 11 and 12, all "country dwellers," were dead, whereas the 4 city dwellers were well and happy. At necropsy all of these animals showed marked injection of the intestinal vessels with no other positive findings, save positive blood cultures in 3 instances, and parenchymatous changes in the viscera.

This surprising result made it necessary to obtain more "country dwellers," if possible, in order to carry out the original plan. Also we endeavored to discover whether the divided dose was to any degree responsible for the apparent sudden increase in the virulence of the B. typhosus, or whether it was a decrease in the normal resistance of

TABLE 3

AGGLUTINATION—FIRST SERIES

Days of Bleeding	"City Dwellers"				"Country Dwellers"	
	1	2	3	5	10	18
Normal: before treatment	—	—	—	—	1:5+	1:5+
1 day after injection of B. typhosus	1:5+	1:5+	—	1:10+	—	—
3 days after injection	1:160+	1:80+	1:80+	1:160+	1:40+	1:40+
6 days after injection	1:2560+	1:320+	1:1280+	1:1280+	1:640+	1:320+
9 days after injection	1:640+	1:320+	1:1280+	1:2560+	1:320+	1:640+
12 days after injection	1:640+	1:80+	1:640+	1:2560+	1:160+	1:160+
17 days after injection	1:160+	1:160+	1:640+	1:320+	1:80+	1:80+
22 days after injection	1:80+	1:80+	1:320+	1:320+	1:80+	1:20+

+ signifies the highest dilution at which definite macroscopic agglutination was obtained. The Stern and Korte dilution method was used.
— signifies no macroscopic agglutination in the highest concentration used (1:5).

our animals. Accordingly, 6 more normal animals (13-18) were injected with B. typhosus, one pair with two 24-hour slant cultures in one dose each, another pair with a divided dose, one culture on the first day and a quarter of a culture on the second; and a third pair with a divided dose of 2 cultures each as in the case of the original series. The first pair receiving the undivided dose died promptly and one each of the other 2 pairs died within 24 hours. Since the surviving member of the third pair had received the same treatment as the original "country dwellers" group, this animal was added to that series as No. 18. The matter of the divided dose was not completely settled, but the evidence pointed to a sudden increase in the virulence of the organism used rather than to the divided dose as the important factor

The 6 rabbits (4 "city dwellers" and 2 "country dwellers") were bled from the ear veins on the 1st, 3rd, 6th, 9th, 12th, 17th and 22nd day after the injection of B. typhosus. The ears were shaved and

treated with tincture of iodin and then alcohol in the usual manner. About 50 drops of blood were collected each time and the serum obtained was preserved in sealed glass tubes in the icebox until all were ready for titration.

The typhosus organism used for agglutination was the same strain as that used in the inoculations. It was grown in large flask cultures on agar and, after 48 hours' incubation, the organisms were suspended in isotonic salt solution and shaken in a mechanical shaker for 8 hours. An excellent homogeneous suspension was thus obtained and the macroscopic method of agglutination was employed with dilutions from 1 : 5 up to 1 : 5120. The same suspension was used for all the titrations. The tubes were incubated for one hour, allowed to stand at room temperature over night, and read the following morning. The serums from each of the "country dwellers" reached the highest point at 1 : 640, while 2 of the city dwellers reached 1 : 2560, one reached 1280, and one 320. The composite curves of the 2 groups are shown in chart 1. The individual figures are given in table 3.

DISCUSSION

Obviously, the striking result in these experiments is the difference in mortality between the "city dwellers" and the "country dwellers" when injected with the same dose of an organism, unrelated to the bacteria previously used for vaccination. Under this condition, the death rate among the "city dwellers" was 20%, whereas the loss among the country dwellers was 83-1/3%. As a partial explanation for this difference in death rate, we find that agglutinins in the group previously vaccinated with cocci attained an average level of 1 : 1360, whereas the average level of 2 unvaccinated animals was 1 : 480. This difference in the agglutinin curve in such a small series of animals would deserve less consideration did it not harmonize with the outstanding difference in the death rate. Aside from furnishing an antibody explanation of the mortality, this correlation between agglutinins and death rate seems to us an indication that agglutinins do not deserve the poor reputation they have been acquiring of late as indicators of immunity.

In repeating these experiments, an attempt was made to approximate more closely the conditions prevailing in the human body by introducing the primary antigen in small daily doses and the secondary antigen by the intratracheal route instead of intravenously. Two sets of rabbits were used, 5 in each group. Animals 1-5 were inoculated

intravenously daily for 21 days with a mixed vaccine made up of Staphylococcus aureus, Staphylococcus albus, Streptococcus buccalis and 2 strains of pneumococci, type undetermined. During the last 2 weeks of this period the vaccines used were attentuated and not completely killed, a slight growth being obtained from a heavy seeding of the control tubes. An amount equivalent to $\frac{1}{10}$th of a slant culture was injected in each instance.

After the period of preliminary vaccination, all the animals were bled from the ear vein about 50 drops each and were injected intratracheally by passing a somewhat blunted needle directly through

TABLE 4

AGGLUTINATIONS—SECOND SERIES

Days of Bleeding	"City Dwellers"			"Country Dwellers"			
	1	3	5	6	7	9	10
After preliminary vaccination; before B. typhosus injection	1:10+	1:10+	1:10+	—	—	—	—
2 days after injection of B. typhosus	1:40+	1:40+	1:10+	—	1:20+	Serum contaminated	1:10+
4 days after injection	1:160+	1:80+	1:80+	1:40+	1:80+	1:40+	1:80+
6 days after injection	1:160+	1:80+	1:160+	1:80+	1:160+	1:40+	1:80+
10 days after injection	1:160+	1:160+	1:320+	1:160+	....	1:40+	1:160+
11 days after injection	1:320+	1:160+	1:160+	1:160+	1:160+	1:40+	1:80+
13 days after injection	1:640+	1:320+	1:80+	1:160+	1:320+	1:[illegible]0+	1:80+
18 days after injection	1:40+	1:80+	1:80+	1:40+	1:320+	1:80+	1:160+
20 days after injection	1:40+	1:40+	1:40+	1:40+	1:160+	1:80+	1:160+

the skin and tracheal wall during ether anesthesia. Eight slant agar cultures of B. typhosus, a sublethal dose, were then forced deeply into the lungs by using a large syringe.

After this injection, animals 1-4 did not appear sick whereas the unvaccinated group showed loss of appetite and appeared generally toxic for about 48 hours. One of the animals, No. 2, died as the result of a mixed infection on the day following the typhosus injection, later No. 4 broke his back during bleeding and No. 8 developed severe ear lesions early in the experiment and was discarded. The remaining animals were bled on the 2nd, 4th, 6th, 10th, 11th, 13th, 18th and 20th days following the injection of B. typhosus (table 4). In this series, we again note a distinct difference in the reaction of the 2 groups, the group previously vaccinated with Gram-positive cocci responding with more than twice the concentration of agglutinins against the secondary antigen, B. typhosus, than did the normal unvaccinated

animals. Neither group of animals in this series produced antibodies to as great a degree as did the intravenous series, but that is to be expected because of the difference in route of injection (Clark and Murphy [14]).

CONCLUSIONS

By the intravenous injections of repeated doses of gram-positive cocci, rabbits are rendered more resistant to the injection of a totally unrelated organism, B. typhosus.

This type of nonspecific vaccination causes the rabbits to respond, when subsequently inoculated with B. typhosus, by building up a higher concentration of agglutinins against this unrelated antigen than do normal animals kept under the same living conditions.

Is it not probable that similar nonspecific immunity may be built up because of the rich and varied bacterial environment of our city life and may be in part responsible for the greater resistance of our city bred students as compared with those reared in rural isolation?

[14] Jour. Infect. Dis., 1922, 31, p. 51.